

CLAIMS

1. A method for determining a target nucleic acid sequence, wherein the target nucleic acid sequence is comprised in a preparation comprising a non-target nucleic acid sequence, the target nucleic acid sequence and the non-target nucleic acid sequence each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the method comprising:

- (a) contacting the preparation with an oligonucleotide primer complementary to at least a portion of the first region of common sequence, under conditions to hybridise the primer thereto;
- (b) contacting the preparation with a first labelled nucleotide bearing a first label, wherein the first labelled nucleotide is complementary to a first template nucleotide comprised in the first region of dissimilar sequence of either the target nucleic acid sequence or the non-target nucleic acid sequence, under conditions to incorporate the first labelled nucleotide either into the primer hybridised to the target nucleic acid sequence or into the primer hybridised to the non-target nucleic acid sequence but not into both;
- (c) subjecting the preparation to a sequencing reaction, thereby extending the primer to form one or more first-labelled sequencing products comprising the first labelled nucleotide and one or more non-first-labelled sequencing products comprising no first labelled nucleotide; and
- (d) determining at least a portion of the sequence of the first-labelled sequencing products and/or the non-first-labelled sequencing products, thereby determining at least the second region of dissimilar sequence of the target nucleic acid sequence.

2. A method according to claim 1, wherein the target nucleic acid sequence and the non-target nucleic acid sequence each have a second region of common sequence which lies between the first and second regions of dissimilar sequence.

3. A method according to claim 1 or claim 2, wherein the method further comprises a step of separating the first-labelled sequencing products from the non-first-labelled sequencing products.
4. A method according to claim 3, wherein the separating step comprises contacting the first-labelled and non-first-labelled sequencing products with a solid phase capable of binding to the first label.
5. A method according to claim 4, wherein the solid phase comprises magnetic beads.
6. A method according to claim 4 or claim 5, wherein the solid phase and the first label together comprise a ligand-affinrant pair.
7. A method according to claim 6, wherein the solid phase comprises streptavidin and the first label comprises biotin.
8. A method according to claim 1 or claim 2, wherein the first label is fluorescent.
9. A method according to any preceding claim, wherein the method comprises a further step of contacting the preparation with a second labelled nucleotide bearing a second label before step (c), the second label being distinguishable from the first label, under conditions such that the non-first-labelled sequencing products but not the first-labelled sequencing products formed in step (c) include the second labelled nucleotide.
10. A method according to claim 9, wherein the second label is fluorescent.
11. A method according to any preceding claim, wherein the first labelled nucleotide is complementary to a first template nucleotide comprised in the first region of dissimilar sequence of the target nucleic acid sequence and the first labelled nucleotide is not complementary to a second template nucleotide at a corresponding position in the non-target nucleic acid sequence.

12. A method according to claim 11, wherein the second labelled nucleotide is complementary to the second template nucleotide.

13. A method for determining a target nucleic acid sequence and a second nucleic acid sequence, comprising a method as defined in any of claims 9 to 12, wherein step (d) comprises determining at least a portion of the sequence of the first-labelled sequencing products and the non-first-labelled sequencing products, thereby determining at least the second region of dissimilar sequence of each of the target nucleic acid sequence and the non-target nucleic acid sequence.

14. A method according to any preceding claim, wherein the first region of dissimilar sequence comprises a single nucleotide.

15. A method according to any preceding claim, wherein the second region of dissimilar sequence comprises a single nucleotide.

16. A method according to any preceding claim, wherein the sequencing reaction comprises a method of sequencing based on the use of dideoxynucleotide terminators.

17. A method according to any preceding claim, wherein the preparation comprises DNA derived from two or more individuals.

18. A method for determining a plurality of target nucleic acid sequences, wherein the plurality of target nucleic acid sequences is comprised in a preparation further comprising a plurality of corresponding non-target nucleic acid sequences, each target nucleic acid sequence in the preparation corresponds to one or more corresponding non-target nucleic acid sequences in the preparation, each target nucleic acid sequence and each corresponding non-target nucleic acid sequence has a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the first region of common sequence of each target nucleic acid sequence is the same as the first region of common sequence of its corresponding non-target nucleic acid sequences, the first region of dissimilar sequence of each target nucleic acid sequence is different to the first region of dissimilar sequence of its corresponding non-target nucleic acid sequences, the second

region of dissimilar sequence of each target nucleic acid sequence is different to the second region of dissimilar sequence of its corresponding non-target nucleic acid sequences, which method comprises:

- (a) contacting the preparation with a plurality of oligonucleotide primers, wherein each primer is complementary to at least a portion of the first region of common sequence of a target nucleic acid sequence and its corresponding non-target nucleic acid sequence, under conditions to hybridise the primer thereto; and
- (b) contacting the preparation with a plurality of first labelled nucleotides wherein each first labelled nucleotide bears a different first label, wherein each first labelled nucleotide is complementary to a first template nucleotide comprised in the first region of dissimilar sequence of a target nucleic acid under conditions to incorporate the first labelled nucleotide into the primer hybridised to the target nucleic acid sequence;
- (c) subjecting the preparation to a sequencing reaction, thereby extending each primer to form one or more first-labelled sequencing products comprising a first labelled nucleotide and one or more non-first-labelled sequencing products comprising no first labelled nucleotide; and
- (d) determining at least a portion of the sequence of each different first-labelled sequencing product and/or each non-first-labelled sequencing product, thereby determining at least the second region of dissimilar sequence of each target nucleic acid sequence.

19. A method according to any preceding claim, wherein the target nucleic acid sequence and the non-target nucleic acid sequence comprise one or more further regions of dissimilar sequence downstream of the second region of dissimilar sequence.

20. A method for determining the haplotype of a subject from a sample comprising DNA from the subject, comprising a method as defined in any preceding claim, wherein the preparation comprises the sample, the target nucleic acid sequence

comprises a locus on a first chromosome of a pair of chromosomes, the second nucleic acid sequence comprises the corresponding locus on the second chromosome of the pair, the locus comprising two or more single nucleotide polymorphisms for which the subject is heterozygous, wherein the sequencing reaction is conducted to determine the sequence of the locus on the first and/or the second chromosome of the pair, thereby determining the haplotype of the subject.

21. A method according to claim 20, wherein the locus comprises a human Class I or Class II HLA gene.

22. Use of pyrosequencing for determining the haplotype of a subject from a sample comprising DNA from the subject, wherein pyrosequencing is used to sequence a target locus on a first chromosome of a pair, the target locus comprising two or more single nucleotide polymorphisms, wherein a labelled nucleotide complementary to a nucleotide of the first single nucleotide polymorphism of one chromosome is selected in order to label all sequencing products derived from one chromosome of a pair.

23. A kit for determining one or more target nucleic acid sequences, wherein the one or more target nucleic acid sequences is comprised in a preparation comprising one or more non-target nucleic acid sequences, the one or more target nucleic acid sequences and the one or more non-target nucleic acid sequences each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, which kit comprises one or more oligonucleotide primers complementary to at least a portion of the first region of common sequence and one or more labelled nucleotides.

24. A kit according to claim 23, wherein the label is fluorescent.

25. A kit according to claim 23 or claim 24, further comprising deoxy-ATP, deoxy-CTP, deoxy-GTP, deoxy-TTP, a DNA polymerase, ATP sulfurylase, firefly luciferase and/or a nucleotide-degrading enzyme.